DeepChek® Assay

CORE Genotyping (RUO)

V1.x

User Guide

24

Version 1 – Revision 1

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

REF **109A24 (old reference: K-16-CORE)**

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Contents

This revision includes changes in the *Assay Components* section.

Application

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The ***DeepChek® Assay CORE genotyping (RUO)*** kit is a single tube system which utilizes PCR technology for amplifying relevant portions of the human C virus (HCV) CORE gene from input RNA extracted from Plasma/serum.

This nucleic acid amplification method might aid in the typing of HCV. This test is NOT intended to be used as a screening or confirmation test for the detection, confirmation and quantification of HCV infection.

The ***DeepChek® Assay CORE genotyping (RUO)*** is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of PCR, SANGER and next generation sequencing (NGS) workflow.

Principles of the assay

The ***DeepChek® Assay CORE genotyping (RUO)*** is a reverse transcriptase-polymerase chain reaction test which includes primers, reverse and forward, designed to amplify HCV RNA from plasma/serum specimens.

First, reverse transcription is performed and the HCV reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded DNA, a second primer anneals to the DNA strand and is extended by the DNA polymerase activity of the enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences.

The ***DeepChek® Assay CORE Genotyping (RUO)*** is performed on a PCR instrument.

Subsequently, the amplicons can be used for SANGER or next generation sequencing and analyzed with a downstream analysis software to list in a report HCV genotypes according to available public reference knowledge databases.

Assay components

The ***DeepChek® Assay CORE Genotyping (RUO)*** is provided in one model of 24 reactions (REF 109A24 / OLD REF K-16-CORE).

Label	Volume for 24 Rxn	Color cap	Storage
RT-PCR Buffer 5X	1 x 320 µL	Green	-25°C to - 15 °C
dNTPs	1 x 65 µL	Brown	-25°C to - 15 °C
RT-PCR Enzyme Mix	1 x 65 µL	Clear	-25°C to - 15 °C
RNAsine	1 x 10 µL	Orange	-25°C to - 15 °C
H_2O	1 x 1500 µL	Blue	-25°C to - 15 °C
CORE FOR Primers	1 x 65 µL	Yellow	-25°C to - 15 °C
CORE REV Primers	1 x 65 µL	Pink	-25°C to - 15 °C
CORE SEQUENCING FOR Primers	1 x 35 µL	Red	-25°C to - 15 °C
CORE SEQUENCING REV Primers	1 x 35 µL	Purple	-25°C to - 15 °C

*Table 1: Volumes and storage conditions of the **DeepChek® Assay CORE (RUO)***

RT-PCR Buffer 5X	dNTPs	RT-PCR Enzyme mix	RNAsine	H_2O
CORE FOR Primers	CORE REV Primers		CORE SEQUENCING FOR Primers	CORE SEQUENCING REV Primers

Picture 1: Mapping of assay components by color cap

Reagent storage and handling

The ***DeepChek® Assay CORE Genotyping (RUO)*** is shipped with dry ice and should be maintained and stored immediately upon receipt at –20°C in order to avoid compromising cold chain integrity.

Expiration date: please refer to the label on the kit box.

Materials required but not provided

- Thermocycler ABI9700
- 96-well plate cooler (Eppendorf / 22510509)
- 96-well PCR plates (Eppendorf / 951020303)
- Plates thermo seals (Thermo Scientific / AB-0558)
- Plate centrifuge
- 0.2 mL thin-wall 8 tubes & domed caps (Thermo Scientific / AB-0266)
- 1.5 mL centrifuge tubes (Dot Scientific Inc. / RN1700-GST)
- Centrifuges tubes (see your specific centrifuge manual)
- Mini centrifuge (see your specific centrifuge manual)
- Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL)
- Ice

Note:

- Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.
- Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

Warnings and precautions

- **For Research Use Only (RUO). Not for use in diagnostic procedures.** No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.
- Laboratories maybe required to report all test results to the appropriate public health authorities.

- Store assay reagents as indicated on their individual labels.
- Do not mix reagents from different kit lots.
- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- Work surfaces and pipettes should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Frequent cleaning of the wells of the PCR instrument plate is recommended to prevent contamination.
- To avoid contamination, use separated and segregated working areas.
- Check whether the PCR reaction tubes are tightly closed before loading on the PCR instrument to prevent contamination of the instrument from leaking tubes.

Starting

- Identify the product.
- Verify the expiration date.
- Verify the latest instruction for use available for the product lot number.
- Verify if the product was used already. If yes, check the remaining tests available.

RNA Extraction

To achieve optimal and sensitive HCV RNA analysis, the best representation of the viral quasispecies, it is recommended to extract **1 mL** of plasma for subsequent cDNA and amplicon generation and elute in the minimum volume required for your preferred extraction kit - MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics) is recommended.

The ***DeepChek® Assay CORE Genotyping (RUO)*** will work with at least an extraction of 400 µL of plasma or serum, ideally from fresh samples, to be eluted in 100µL (related sensitivity evaluated to 1250 UI/mL).

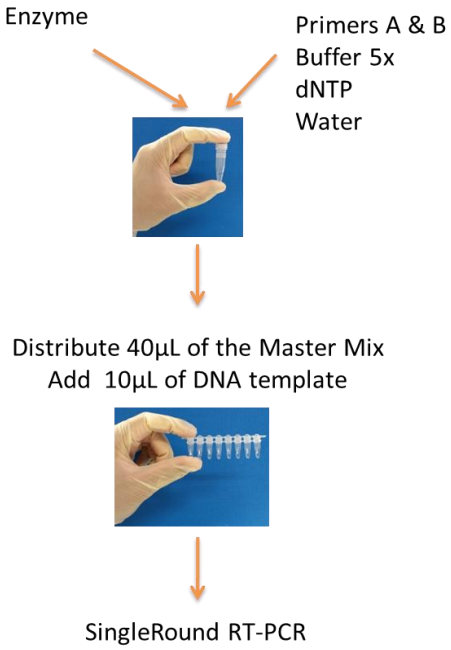
For samples with low viral load, we recommend:

1. To perform an ultracentrifugation procedure. Pellet the virus for 1.5 hours at 40,000 g (or alternatively for 2 hours at 24,000g), and at 4°C. Remove enough supernatant to leave the required amount of sample for your preferred extraction kit.

OR

2. To extract one or 2 mL of plasma/serum and elute in the minimum volume required for your preferred extraction kit.

SingleRound RT-PCR Workflow Overview



SingleRound RT-PCR Step-by-Step Workflow for CORE Target

1. Thaw extracted template RNA, primer solutions, dNTP Mix, 5x Buffer and RNase-free water and place them on ice. Load all the tubes into the centrifuge. Spin the samples at 11000 g for 10 seconds. Then aspirate and discharge the solution several times before the dispensing.
2. Prepare a master mix according to **Table 2**. The master mix typically contains all the components required for RT-PCR except the template RNA. Prepare a volume of master mix greater (n+1) than that required for the total number of reactions to be performed.

Reagent	Volume
Buffer 5X	10 µL
dNTP 10 mM	2 µL
CORE FOR Primer 10 µM	2 µL
CORE REV Primer 10 µM	2 µL
RT-PCR Enzyme Mix	2 µL
RNAsine	0.15 µL
H_2O	21.85 µL
Final volume	**40 µL**

Table 2: Reaction components for SingleRound RT-PCR CORE target

3. Vortex the master mix thoroughly, spin down and dispense 40 µL into PCR tubes. Keep the tubes on ice.
4. Add 10µL of RNA to the PCR tubes. Mix by pipetting the master mix up and down a few times.
5. Program the thermal cycler according to the program in **Table 3**.

Cycle	Temperature (°C)	Time
1	50	40 min
1	95	15 min
50 cycles	94	30 sec
	64	30 sec
	72	1 min 10 sec
1	72	10 min
1	10	∞

Table 3: SingleRound RT-PCR CORE Cycling Program

6. Start the DeepChek® SingleRound RT-PCR and Sequencing CORE cycling program while PCR tubes are still on ice. **Wait until the thermal cycler has reached 50°C. Then place the PCR tubes in the thermal cycler**. After amplification, samples can be stored overnight at 2–10°C or at - 20°C for long-term storage.
7. **[Recommended]** – RT-PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of the signal. Even if low-intensity bands usually lead to a successful sequencing, it is recommended to avoid the process if no band can be observed.

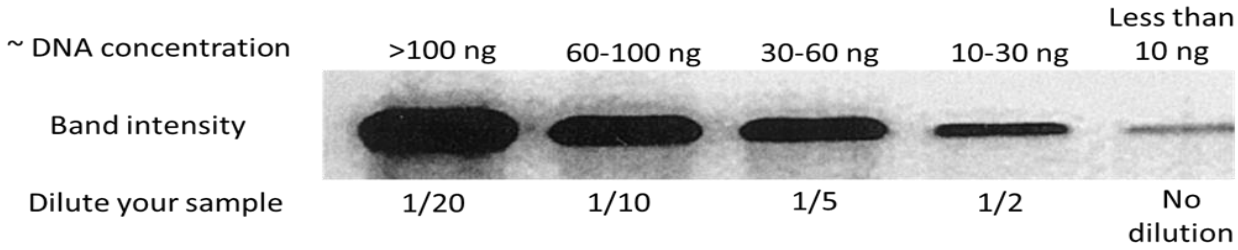
Expected amplicons size: CORE (463 bp)

SingleRound RT-PCR Troubleshooting Guide

1. Check the concentration, storage conditions and quality of the starting template. For optimal results use fresh/frozen plasma/serum and proceed with fresh RNA extraction.

2. For samples with low viral load, we recommend to perform an ultracentrifugation procedure. Pellet the virus for 1.5 hours at 40,000 g and at 4°C. Remove enough supernatant to leave the required amount of sample for your preferred extraction kit.

3. In presence of very large PCR bands on the agarose gel, dilute (1/101 - 1/103) of the product before sequencing.



PCR Products Purification

Before sequencing, first make sure your RT-PCR products have been purified. In presence of very large PCR bands on the agarose gel, dilute ($1/10^1$ - $1/10^3$) of the SingleRound RT-PCR product before sequencing.

Sequencing

1. Purification

For amplicons greater than 500bp, use a 0.6x AMPure XP cleanup (30 µl volume of beads). For amplicons less than 500bp, use a 1.8x AMPure XP cleanup (90 µl volume of beads) to maximize yield. Or use PCR Clean Up.

2. Sanger sequencing

In presence of very large PCR bands on the agarose gel, make dilution ($1/10^1$ - $1/10^3$) of the SingleRound RT-PCR product before sequencing.

For CORE sequencing, use the red and purple cap tubes containing 35µL of each CORE Sanger sequencing primer (forward and reverse - 3.2 µM). **1µL** of each primer will be used for each sample.

1. Prepare the sequencing reaction according to the **Table 4a** (Big Dyes Terminator kit v1.1) or **4b** (Big Dyes Terminator kit v3.1).

Reagent for *Forward* Sequencing	**Volume**
Big Dye Terminator v1.1	1 µL
Sequencing Buffer	1 µL
Forward Primer (3,2µM)	**1 µL**
Purified RT-PCR	0.7 – 2 µL
Water	q.s. to 10 µL

Reagent for *Reverse* Sequencing	**Volume**
Big Dye Terminator v1.1	1 µL
Sequencing Buffer	1 µL
Reverse Primer (3,2µM)	**1 µL**
Purified RT-PCR	0.7 – 2 µL
Water	q.s. to 10 µL

Table 4a: Sequencing reaction for Big Dyes Terminator kit v1.1

Reagent for *Forward* Sequencing	**Volume**
Big Dye Terminator v3.1	2 µL
Sequencing Buffer (5x)	3 µL
Forward Primer (3,2µM)	**1 µL**
Purified RT-PCR	0.7 – 2 µL
Water	q.s. to 15 µL

Reagent for *Reverse* Sequencing	**Volume**
Big Dye Terminator v3.1	2 µL
Sequencing Buffer (5x)	3 µL
Reverse Primer (3,2µM)	**1 µL**
Purified RT-PCR	0.7 – 2 µL
Water	q.s. to 15 µL

Table 4b: Sequencing reaction for Big Dyes Terminator kit v3.1

2. Program the thermal cycler according to the program in **Table 5a** (Big Dyes Terminator kit v1.1) or **5b** (Big Dyes Terminator kit v3.1).

Cycle	**Temperature (°C)**	**Time**
1	96	5 min
25 cycles	96	10 sec
	50	5 sec
	60	4 min
1	4	∞

Table 5a: Thermal cycler for Big Dyes Terminator kit v1.1

Cycle	**Temperature (°C)**	**Time**
1	96	1:30 min
25 cycles	96	20 sec
	50	15 sec

	60	1:30 min
1	4	∞

Table 5b: Thermal cycler for Big Dyes Terminator kit v3.1

3. **Sephadex purification** - Complete all the sequencing reaction with water (q.s. to 20µL). Purify all sequencing reaction (20µL) with Sephadex gel before the final Sanger sequencing.

OR

3. **BigDye XTerminator® Purification kit.**

OR

3. **Ethanol purification**

a) Add 4ul of EDTA 125mM and sodium acetate 3M (1:1) solution;
b) Add 50ul Ethanol 100%;
c) Seal the plate well and gently vortex;
d) Incubate at room temperature (protected from light) for 15 minutes; While the plate is incubating, change the temperature of the centrifuge to 4ºC;
e) Centrifuge the plate at 4ºC for 35 minutes, at 4000rpm;
f) Remove the plate immediately, remove the sealing, and place the plate face down on a paper towel.
g) Briefly centrifuge the place face down until 185rpm;
h) Add 55ul of Ethanol 70%;
i) Seal the plate well and vortex for 15 seconds;
j) Centrifuge the plate at 4ºC for 15 minutes, at 4000rpm;
k) Remove the plate immediately, remove the sealing, and place the plate face down on a paper towel;
l) Centrifuge the place face down for 1 minute at 4000rpm;
m) Place the plate at 94ºC for maximum 1 minute.

4. **Denaturation:** Add 10 µL of formamide and incubate at the thermocycle at 94°C for 5 minutes then immediately incubate the plate at 4°C for thermal shock for at least 5 minutes.

3. NGS

After the amplicon verification, the samples are ready for the NGS kit processing:

Through Illumina MiSeq

- **116AX or 116BX | DeepChek® NGS LIBRARY PREPARATION (24 / 48 or 96 reactions).**
- MS-103-1003 | MiSeq Reagent Nano kit, v2 (500 cycles).

Through Ion Torrent

- **4471269** | Ion Xpress™ Plus Fragment Library Kit
- **4471250** | Ion Xpress™ Barcode Adapters 1-16 Kit
- **4484355** | Ion 318™ Chip Kit v2

Data Analysis

1. Sanger

AB1 or FASTA files containing nucleotide sequences for CORE are analyzed by the **DeepChek®** software. User shall then follow the DeepChek software procedure to complete the data analysis and reporting processes.

2. NGS

NGS files containing nucleotide sequences for CORE are analyzed by the **DeepChek®** software. User shall then follow the DeepChek software procedure to complete the data analysis and reporting processes.

Product quality control

In accordance with ABL's Quality Management System, each lot of the assay is tested against predetermined specifications to ensure consistent product quality. Certificates of Analysis are available upon request.

Symbols

 <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution		Temperature limitation
	Catalog number		Serial Number
	Use by	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Manufacturer		Country of manufacturing
	Distributor		

Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: https://ablsa.odoo.com/fr_FR/issue; Email: support-diag@ablsa.com; Call +339 7017 0300 Or contact your ABL Field-Application Specialist or your local distributor. For up-to-date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide. ABL User Guides are available at **www.ablsa.com/ifu** or can be requested from ABL Technical Services or your local distributor.

Manufacturer

Advanced Biological Laboratories (ABL) S.A.

52-54 Avenue du X Septembre, L-2550 Luxembourg, Luxembourg

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument. The information in this guide is subject to change without notice. DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, ABL (S.A) AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Version 1.1

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